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(54) Somatostatin analogs to specific delivery of anti-tumor drugs into tumor cells

(57) The present invention is directed to a novel cancer therapeutic compound having a general formula:

paclitaxel (or PEG-DOPE)-O-R-NH-R'

wherein R is a di-carboxylic acid and R' is a somatostatin analog.

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Description

[0001] The present invention relates to a novel anti-cancer drug delivery system, wherein a somatostatin analog is conjugated with an anticancer drug, such as paclitaxel, so that the anticancer drug can be delivered specifically to the targeted cancer cells.

[0002] Most cytotoxic anticancer drugs suffer from a common problem, i.e. toxic side effects due to the lack of a selective drug delivery system. Using endocytotic ligands as carriers of the anticancer drugs to target these drugs to the cancer cells can avoid toxic side-effects and greatly improve the efficiency of these drugs' delivery.

[0003] Somatostatins function through cellular membrane receptors, known as somatostatin receptors (SSTR). These receptors are over expressed on the surfaces of certain tumor cells, such as carcinoid, islet cell of the pancreas, paragangliomas and small-cell carcinomas of lungs. Since somatostatin analogs possess such interesting properties, they may be used as a carrier system targeted to those malignant tumor cells such that the drugs can specifically act on those cells. It has been reported that somatostatin analogs including X-c[Cys-Phe-Trp-Lys-Thr-Cys]-X, X-c[-Cys-Tyr-D-Trp-Lys-Val-]-X, or X-c[Cys-Phe-D-Trp-Lys-Thr-Cys]-X are labeled with chromatic or radionuclide to visualize and monitor the tumors that express SSTRs. Somatostatin analogs are particularly preferred for these applications to the original somatostatins because such analogs are known to be smaller in size, higher in affinities to the somatostatin receptors, and more stable than the original somatostatins.

[0004] The present invention is directed to a combination of somatostatin analogs, such as octreotide, lanreotide, and vapreotide, and a cytotoxic drug, such as paclitaxel, through a covalent bond or a physical encapsulation. The inventive compound of the present invention has the following general structure:

X-O-spacer-NH-peptide

wherein X is an anticancer drug, a lipid for making a liposome, or a monomer for forming a polymer matrix.

[0005] In general, this invention employs somatostatin analogs having sequence of X-c[Cys-Phe-Trp-Lys-Thr-Cys]-X, X-c[-Cys-Tyr-D-Trp-Lys-Val-]-X, or X-c[Cys-Phe-D-Trp-Lys-Thr-Cys]-X to provide a new system for delivering anticancer drugs to the cancer cells. The synthesis of somatostatin analogs are performed on a solid-phase peptide synthesizer using Fmoc Chemistry. The desired compound, such as anticancer drugs paclitaxel, doxorubicin or camptothecin or the like, that is intended to be delivered to the target cells, reacts with a spacer having a carboxyl terminal group to form a drug-spacer complex. Such complex is then coupled to the N-terminal of the somatostatin analog peptide on the resin to form the final product, namely, drug-spacer-peptide, as shown in Fig, 1.

The present invention further provides a method of synthesizing a DOPE-PEG-spacer-somatostatin analog complex, which is useful for the preparation of a liposome drug delivery system, as shown in Fig. 1.

[0006] The various features of novelty which characterize the invention are pointed out with particularity in the claims annexed to and forming a part of the disclosure. For a better understanding of the invention, its operating advantages, and specific objects attained by its use, reference should be had to the drawing and descriptive matter in which there are illustrated and described preferred embodiments of the invention.

[0007] In the accompanying drawings:

Fig. 1 shows general schemes of forming somatostatin analog conjugates and the sequences of the three preferred somatostatin analogs;

Fig.2 are fluorescence micrographs of MCF-7 and CHO cells after incubation with fluorescin-octreotide at 4°C for 30 min. The labeling is confined to the MCF-7 cell surface (B) and disappears when the MCF-7 cells were incubated in the presence of an excessive amount (1,000 fold) of nonfluorescent octreotide (D). No labeling can be seen among the CHO cells (F), even these cells were incubated with a higher concentration (500 μg/ml) of fluorescin-octreotide (H). Cell morphology was shown at A, C. E, and G. Bar=20 μm;

Fig.3 shows the internalization of fluorescin-octreotide by the MCF-7 cells. Bar=5μm.

Fig.4 shows that The octreotide-conjugated taxol virtually retains the cellular functions of taxol.

Fig. 5 shows that the octreotide-conjugated paclitaxel is specific to MCF-7 cells.

[0008] The abbreviations:

Fmoc: 9-fluorenylmethoxycarbonyl
Trt: triphenylmethyl
Thr-ol: threoninaol
Phe: phenylalanine
Cys: cysteine

Thr: threonine
Lys: lysine
Trp: tryptophan
DβNal: D-β-(2-naphthyl) alanine
TFA: trifluoroacetic acid
DMF: N,N-dimethylformamide
THF: tetrahydrofuran
HBTU: 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
DMAP: 4- dimethylaminopyridine
ACN: acetonitril
DCM: dichloromethan
TIS: Triisopropylsilane
PEG: polyethylene glycol
DOPE: dioleoyl phosphatidyl ethanolamine
MTT: 4,5-5-dimethyl thiazol-2,5-diphenyl-terasolium bromide

[0009] The formation of stable, covalently linked conjugates with fully retained biological activities of the anticancer drugs is achieved by using a di-carboxylic acid spacer, such as glutaric acid. One carboxyl group of the spacer forms an ester bond with the 2'-OH group of anticancer drug paclitaxel or the -OH groups of other anticancer drugs and the other carboxyl group of the spacer forms a carboxamide bond with a well chosen free amino group of the peptide carrier, such as a somatostatin analog. While all somatostatin analogs that have free N-terminal amino groups may be chosen to conjugate with the anticancer drug or the like through the spacer, the particularly preferred peptide carriers are somatostatin analogs octreotide, lanreotide and vapreotide for their high affinities to human somatostatin receptors subtype 2 (for octreotide), 5 (for lanreotide) and 4 (for vapreotide). The sequences of the three preferred somatostatin analogs are shown as follows:

octreotide: D-Phe-c[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr(ol)
lanreotide: DβNal-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Thr-NH_2
vapreotide: D-Phe-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Trp-NH_2

[0010] The somatostatin analogs used for the purpose of the present invention may be synthesized using techniques known in the art, extracted from natural systems, or obtained from commercial sources (e.g. Peninsula, Neosystems, Sigma and BASF). A list of somatostatin analogs which may be used is described, for example, in "Somatostatin", Weil, Muller, and Thorner (eds.) (1992), the contents of which are incorporated herein by reference. Preferably, the somatostatin peptide is synthesized using conventional solid-phase synthetic techniques.

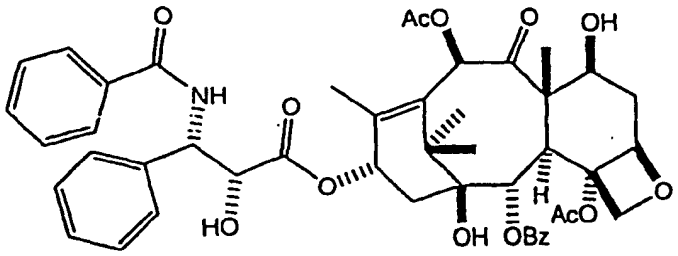
[0011] According to the present invention, the desired compound X is linked with one of the two carboxyl groups of a dicarboxylic acid spacer R and the somatostatin analog with the other, which results in a general formula represented by:

X-O-R-NH-R'

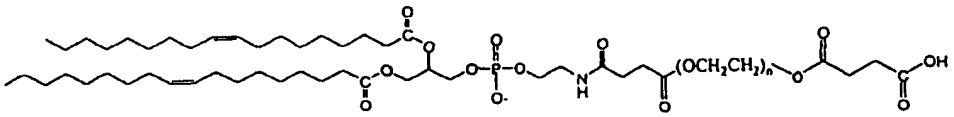
wherein-R- is -C(O)-$(CH_2)_n$-C(O)- and n=0-7, and R'- is a somatostatin peptide moiety.

[0012] It is contemplated that compounds having general formulas of NH_2-$(CH_2)_n$-COOH, or compounds NH_2-(PEG)-COOH, HOOC-(PEG)-COOH or HOOC-X-Maleimide may also be used as a spacer as long as somatostatin analogs are extended their length at N-terminal with amino acid derivatives which possess free thio, carboxyl moieties or the like.

[0013] The desired compound X may be the anticancer drug paclitaxel, which has the formula



or PEG-DOPE known for its capability of forming liposomes under the condition specified below, PEG-DOPE having the formula



[0014] Besides the above-identified compounds, any other compounds capable of forming an ester bond with the carboxyl group of the spacer may also be used for the purpose of the present invention.

[0015] Paclitaxel may be obtained from a commercial source (e.g. Bristol-Myers-Squibb), purified from a natural source or chemically synthesized using techniques known in the art. Both PEG and DOPE are generally available.

[0016] Once the drug-somatostatin conjugates are formed through the spacers, their biological activities can be tested using cell culture techniques.

[0017] Referring now to Fig. 2, specific binding of octreotide to the MCF-7 cells but not the CHO cells is shown in fluorescence micrographs. panels A and C, and E and G show the morphology of the MCF-7 cells and CHO cells. Panels B, D, F and H which correspond to panels A, C, E and G, respectively, show the fluorescence labeled octreotide binding to the MCF-7 cells and the CHO. The MCF-7 cells and CHO cells are inclubated with 100 μg of fluorescein labeled octerotide in 1 ml of a buffer for 30 minutes at 4 °C, after which the cells are washed with PBS, fixed with 4% paraformaldehye and subject to fluorescence microscopy. As shown in panels B and F, the octreotide binds to the MCF-7 cells (B) but not to the CHO cells (F). The octreotide binding to the MCF-7 cells disappears in the presence of an excessive non-labeled octreotide to compete with the labeled octreotide (panel D). The octreotide does not bind to the CHO cells even when the CHO cells are given a higher concentration of labeled octreotide (panel H).

[0018] Fig. 3 shows that the labeled octreotide bound to the MCF-7 cells was internalized into the MCF-7 cytosol via somatosatin receptor-mediated endocytosis after one hour incubation with 100 μg/ml of the labeled octreotide at 37 °C.

[0019] Referring now to Fig. 4, the octreotide conjugated paclitaxel exhibits the same antitumor activity through disrupting microtubule formation of the cells as the non-conjugated paclitaxel. Panel A shows distribution of tubulin in the MCF-7 cells, where the cells were incubated in the absence (A1) or presence (A2) of 10^{-6} paclitaxel, or in the presence of octreotide-conjugated paclitaxel (A3). Bar=20μm. B and C: Chromatin condensation in apoptotic cells, where the ultrastructure of apoptotic MCF-7 cells were observed by transmission electron micrograph (B, X 7,500, bar = 500 nm) or the Nuclei were stained with Hoechst 33258 and fluorescence photomicrographs (C, bar = 10 μm); the MCF-7 cells being treated without (B1 and C1) or with (B2 and C2) 10^{-6} paclitaxel or with octreotide-conjugated paclitaxel (B3 and C3) for 1 day.

[0020] Cell-specificity of the octreotide-conjugated paclitaxel is shown in Fig. 5, where panels A1 and B1 are the untreated MCF-7 cells and CHO cells, respectively; panels A2 and B2 are MCF-7 cells and CHO cells, respectively, treated with paclitaxel (10^{-5}M) for 1 day; and panels A3 and B3 show the MCF-7 cells and CHO cells, respectively, treated with the octreotide conjugated paclitaxel (10^{-5}M) for 1 day. Unlike the free paclitaxel, which causes death of both MCF-7 cells and CHO cells, the octreotide conjugated paclitaxel induces only the death of the MCF-7 cells but not of the CHO cells as shown in panels A3 and B3. Cell death is indicated by arrows, bar = 20 m.

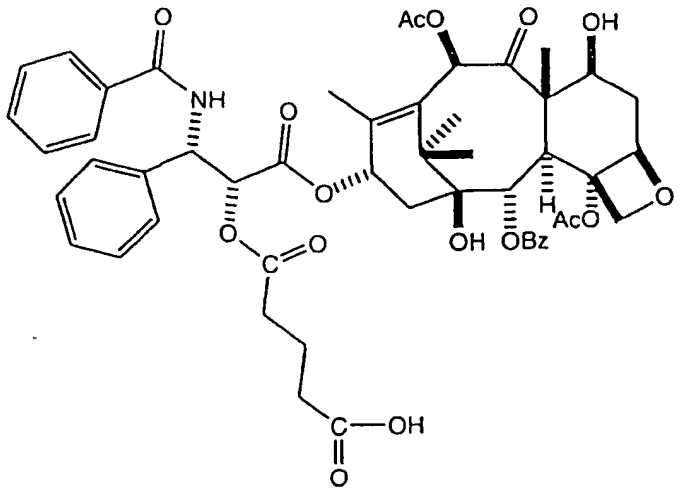
[0021] The novel somatostatin-conjugated compounds of the present invention may be used for treating a cancer patient by administering the anticancer compounds to the cancer patient in a composition comprising the above described compounds and a pharmaceutically acceptable carrier.

[0022] The practice of the invention is further illustrated by the following examples, but the illustration does not limit the scope of this application.

Example 1

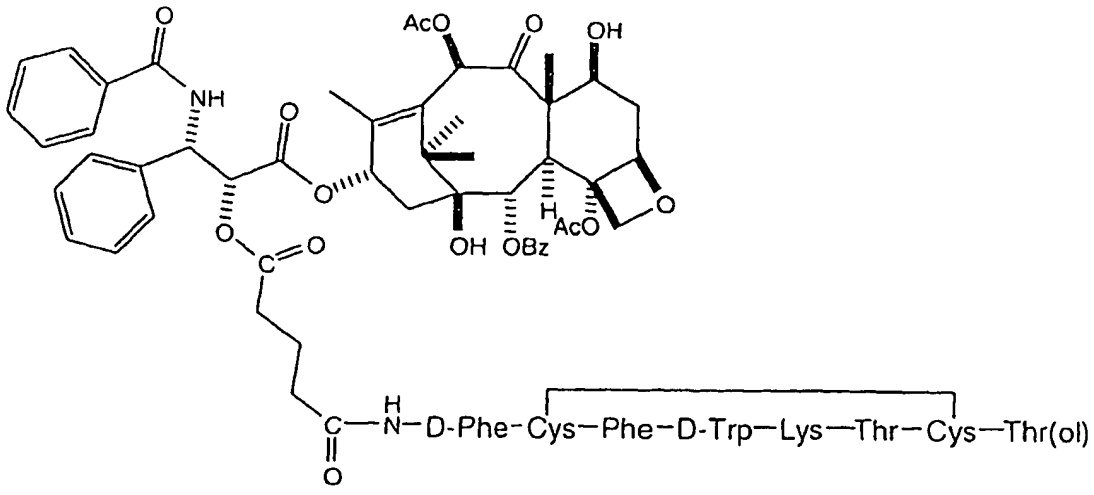
Synthesis of Paclitaxel-Glutary 1-Octreotide

[0023] Paclitaxel (0.43g, 0.5 mmol) and glutaric anhydride (0.68g, 6 mmol) were dissolved in 5 ml of pyridine and stirred at room temperature for 3 hours. In the end of reaction, the solution was evaporated to dryness *in vacuo*. The residues were treated with 20 ml water with stirring for 20 min and filtered. The precipitates were redissolved in acetone and water was added to produce fine crystals of paclitaxel-glutarate (0.42g, 86% yields). The analytic result gave $[M+H]^+$ = 968 Da by ESMs. The paclitaxel-glutarate complex has the following structure:



[0024] The sequence of octreotide was synthesized using solid-phase Fmoc chemistry, which has an assembly chain of NH_2-D-Phe-Cys(Trt)-Phe-D-Trp-Lys(Mtt)-Thr-Cys(Trt)-Thr-ol-Acetal-Siber Amide Resin (0.1 mmol). Four molar equivalent of paclitaxel-glutarate activated by HBTU was added as the ninth amino acid derivative to couple octreotide. The final of reaction was monitored using ninhydrine test that measures the diminished free amino group at N-terminal.

[0025] Cleavage of the peptide conjugate from amide resin was performed using 1% TFA/5% TIS/DCM. The cleaved compounds were neutralized by 15% pyridine/methanol and diluted with pH 7.5 buffer to complete disulfide-formation and lyophilized to 135 mg of the crude product. Further purification may be performed by HPLC with a preparative column and ACN/H_2O eluent system. The analytic result gave $[M+H]^+$=1969 Da by ESMs and following structure:



Example 2

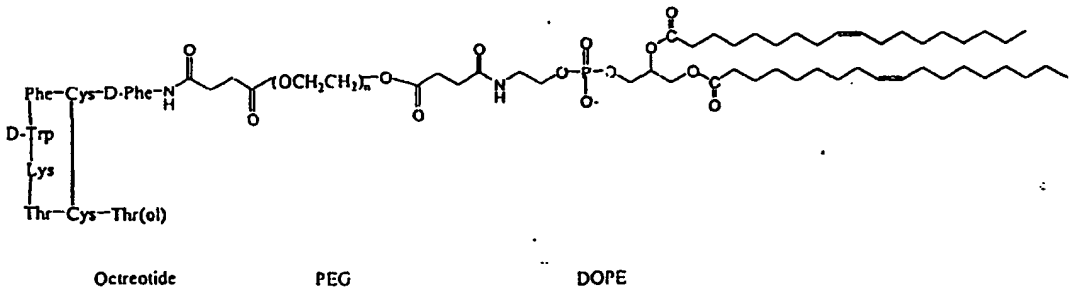
Synthesis of Fluorescein-Octreotide

[0026] Four equivalent molar of 5-Carboxy-fluorescein (0.4 mmole) were coupled to the assembly chain of NH_2-β-Ala-D-Phe-Cys(Trt)-Phe-D-Trp-Lys(Mtt)-Thr-Cys(Trt)-Thr-ol-Acetal-Siber Amide Resin (0.1 mmol) as an amino acid derivatives. Cleavage of the peptide conjugate from amide resin was performed using 95% TFA/5% TIS. After evaporation of TFA under vacuum, the peptide was precipitated with addition of ice cold dry ether. The precipitates were filtered, washed with cold ether on a sintered glass funnel, and extracted with 20% acetic acid solution. The peptide product was diluted to about lmM with 5% ammonium acetate solution and the pH was adjusted to 7.5 with ammonium hydroxide (25%) to accomplish disulfide-formation. The sample was then lyophilized to powder. The analytic result gave $[M+H]^+$ m/z 1448 Da by ESMs.

Example 3

Synthesis of DOPE-PEG-Octreotide

[0027] PEG (1g, 0.5 mmole) and succinic anhydride (0.1g, 1mmole) were dissolved in THF with addition of DMAP. The solution was stirred at 50 °C with reflux for six hours. In the end of reaction, the solution was evaporated *in vacuo* to produce di-succinyl-PEG, which was purified by flash chromatography of the residue over silica gel. Four equivalent molar of di-succinyl-PEG(0.4 mmole) and four equivalent of DOPE (0.4 mmole) were coupled to the assembly chain of NH_2-D-Phe-Cys(Trt)-Phe-D-Trp-Lys(Mtt)-Thr-Cys(Trt)-Thr-ol-Acetal-Siber Amide Resin (0.1 mmol) sequentially as the ninth and the tenth of amino acid derivatives. Cleavage of the peptide conjugate from amide resin was performed using 1% TFA/5% TIS/DCM. The cleaved compounds were neutralized with 15% pyridine/methanol, diluted with a buffer of pH 7.5 to complete disulfide-formation, and lyophilized to crude product having the structure below. This product was used in the liposome preparation.



Example 4

Liposome Preparation

[0028] Liposome was prepared by lipid/detergent mixed micelles followed by controlled dialysis. A solution of DSPC/cholestrol/DOPE-PEG-octreotide (molar ratio 10:1:1) in 10 ml chloroform/ethanol (2:1 v/v) was mixed with sodium cholate (lipid/cholate molar ratio 0.6) and evaporated to dryness under reduced pressure in a round-bottom flask at 55 °C. The remaining thin film was dispersed in 5 ml 10 mM phosphate buffer pH 7.4 which was adjusted to 0.16 ionic strength. The mixed micelles were spontaneously formed. After short equilibration, the mixed micellar solution was dialyzed at 60 °C for 24 hours with the MINI-LIPOPREP^R (Sialomed, Inc., USA) using cellulose disk membranes having a 10,000 molecular weight cut off. The liposome so prepared was directly used for assay.

[0029] The invention is not limited by the embodiments described above which are presented as examples only but can be modified in various ways within the scope of protection defined by the appended patent claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Wu, Ying-Ta
Huang, Chun-Ming
Chen, Shui-Tein

(ii) TITLE OF INVENTION: Application of Somatostatin Analogs to Specific Delivery of Antitumor Drugs into Tumor Cells

(iii) NUMBER OF SEQUENCES: 3

(iv) CORRESPONDENCE ADDRESS:
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(C) CITY: New York
(D) STATE: New York
(E) COUNTRY: U.S.A.
(F) ZIP: 10176

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.5" floppy disk
(B) COMPUTER: IBM
(C) OPERATING SYSTEM: Microsoft Window 95
(D) SOFTWARE: Microsoft Word

(2) INFROMATION FOR SEQ ID NO: 1
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: cyclic

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCREPTION: SEQ ID NO: 1

D-Phe-c[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr(ol)
1 5

(2) INFROMATION FOR SEQ ID NO: 2

(iii) SEQUENCE CHARACTERISTICS:
(E) LENGTH: 8 amino acids
(F) TYPE: amino acid
(G) STRANDEDNESS: not relevant
(H) TOPOLOGY: cyclic

(iv) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCREPTION: SEQ ID NO: 2

DβNal-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Thr-NH_2
1 5

(2) INFROMATION FOR SEQ ID NO: 3
(v) SEQUENCE CHARACTERISTICS:
(I) LENGTH: 8 amino acids
(J) TYPE: amino acid
(K) STRANDEDNESS: not relevant
(L) TOPOLOGY: cyclic

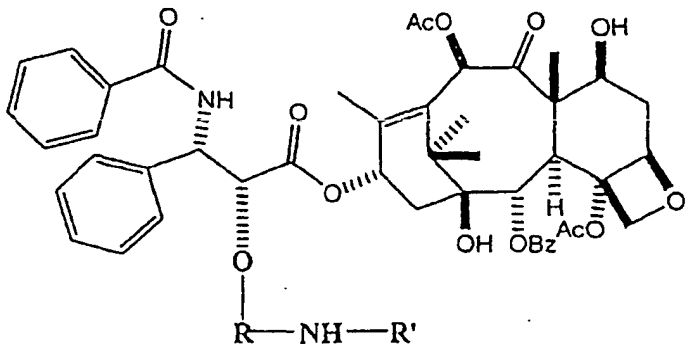
(vi) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCREPTION: SEQ ID NO: 3

D-Phe-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Trp
1 5

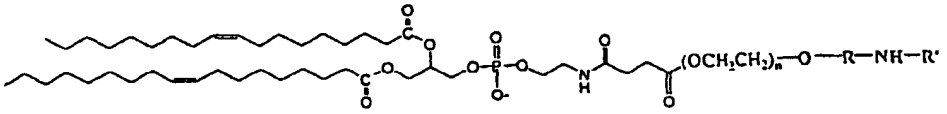
Claims

1. A compound of formula:



wherein -R- is -C(O)-$(CH_2)_n$-C(O)-, n is an integer of from 0 to 7, and R' is a somatostatin-analog peptide moiety.

2. A compound of formula:



wherein -R- is $-C(O)-(CH_2)_n-C(O)-$, n is an integer of from 0 to 7, and R' is a somatostatin-analog peptide moiety.

3. The compound of claim 1 or 2, wherein said somatostatin-analog peptide is octreotide (SEQ ID NO: 1), lanreotide (SEQ ID NO: 2) or vapreotide (SEQ ID NO: 3).

4. The compound according to any one of claims 1 to 3, wherein n=2.

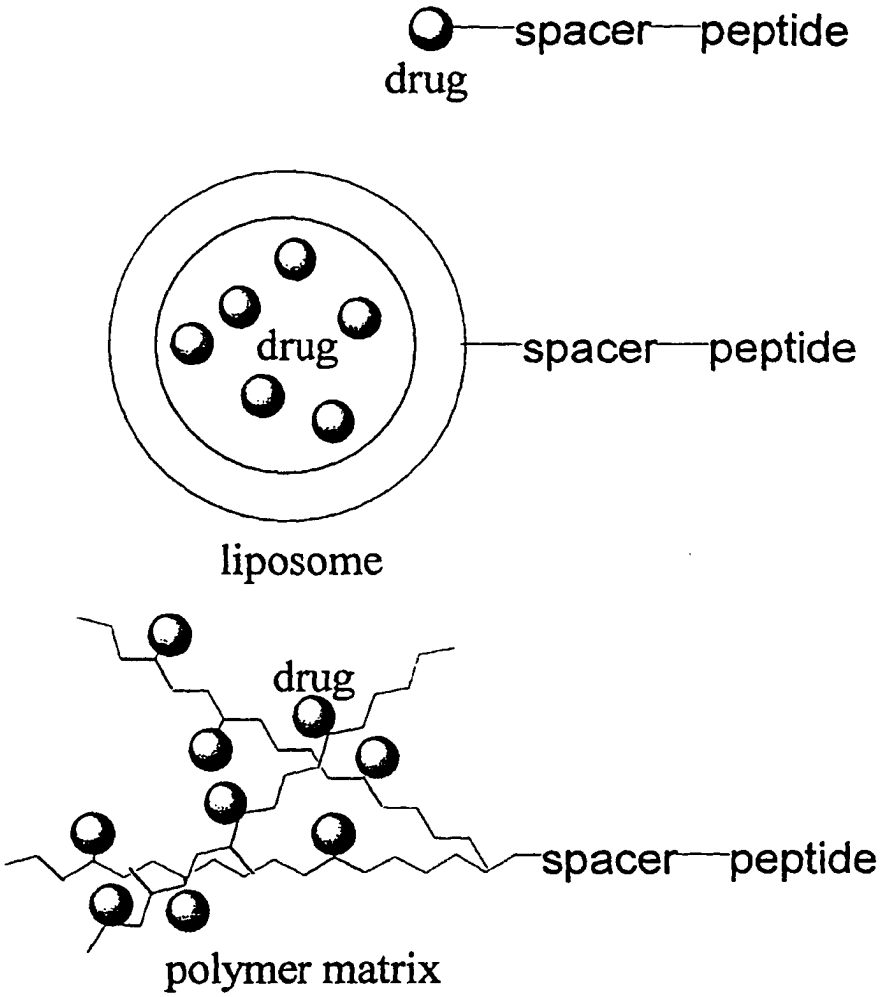
5. A liposome comprising a compound according to any one of claims 2 to 4.

6. A pharmaceutical composition comprising a compound according to any one of claims 1 to 4 or a liposome according to claim 5 and a pharmaceutically acceptable carrier or diluent.

7. A compound according to any one of claims 1 to 4 or a liposome according to claim 5 for use in a method of treatment of the human or animal body by therapy.

8. A compound or liposome according to claim 7 for use in a method of treatment of cancer.

9. Use of a compound according to any one of claims 1 to 4 or a liposome according to claim 5 in the manufacture of a medicament for use in the treatment of cancer.



Peptide = D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr(ol) (Octreotide)

DβNal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 (Lanreotide)

D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2 (Vapreotide)

FIG.1

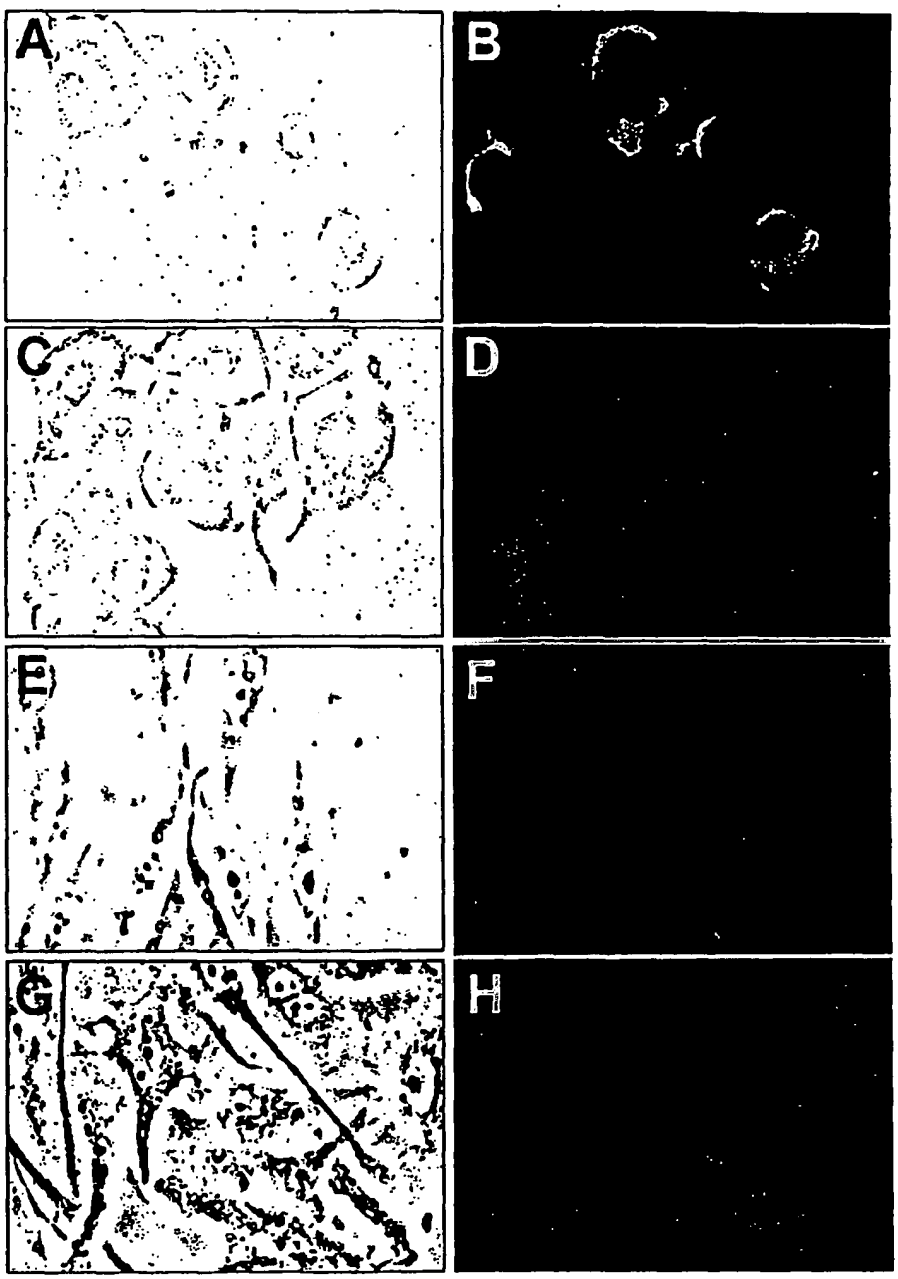


FIG.2

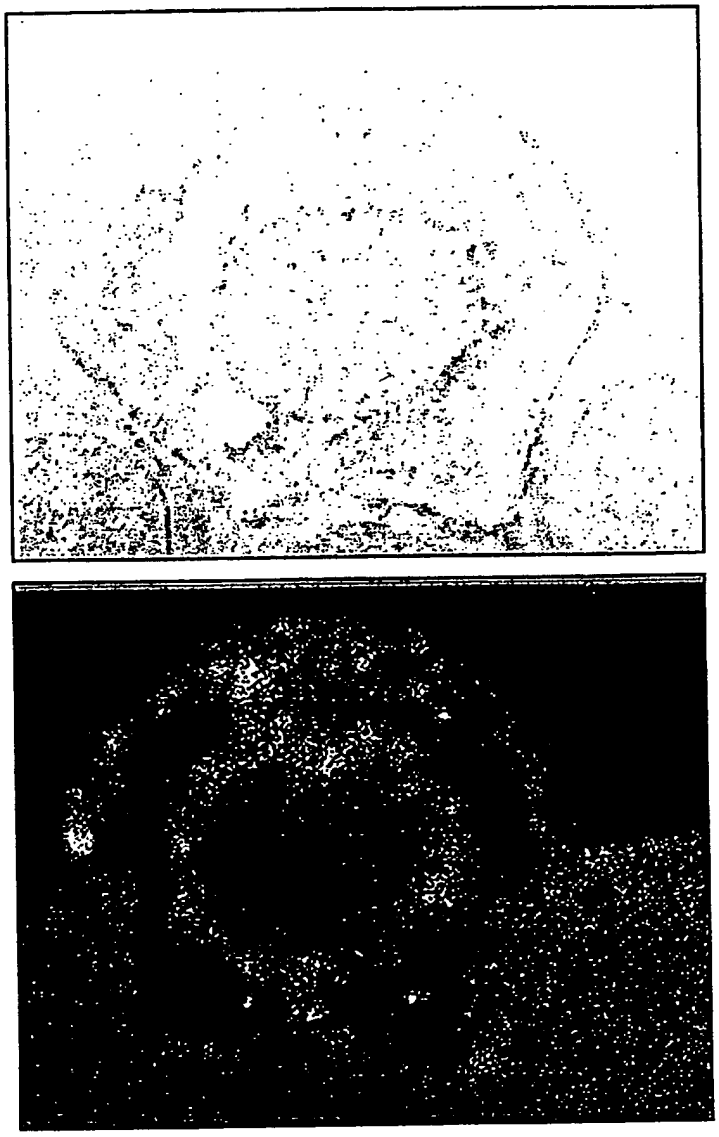


FIG.3

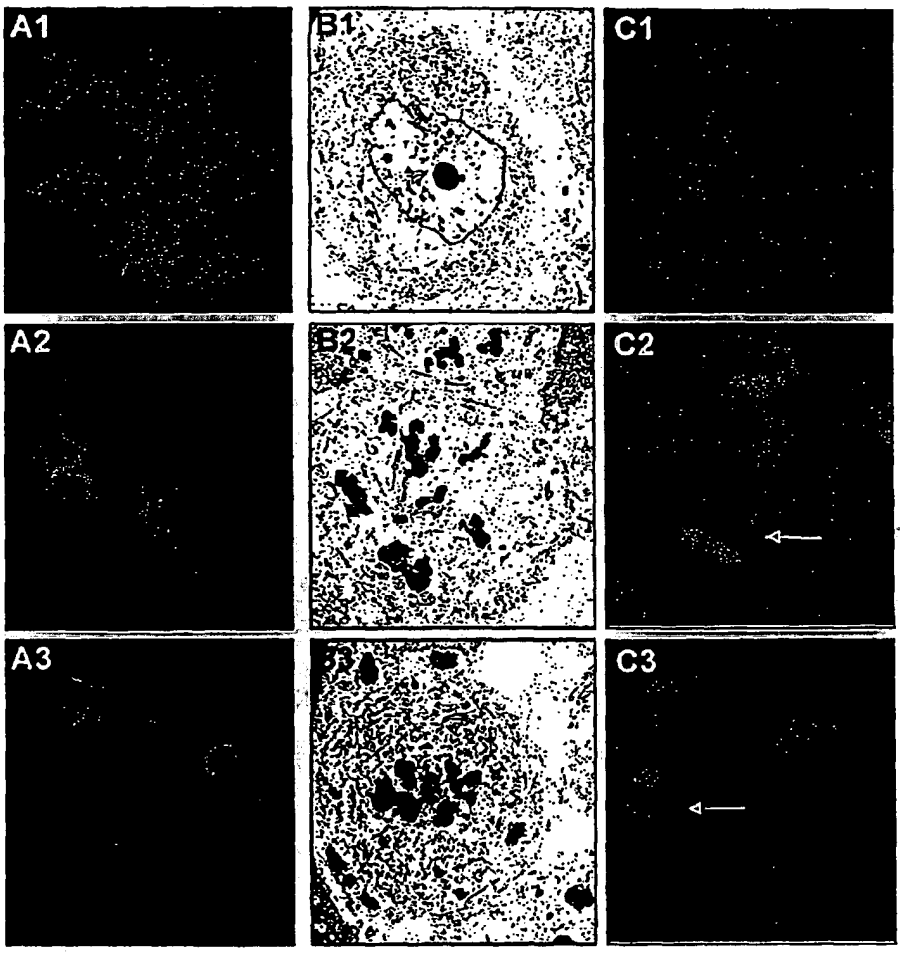


FIG.4

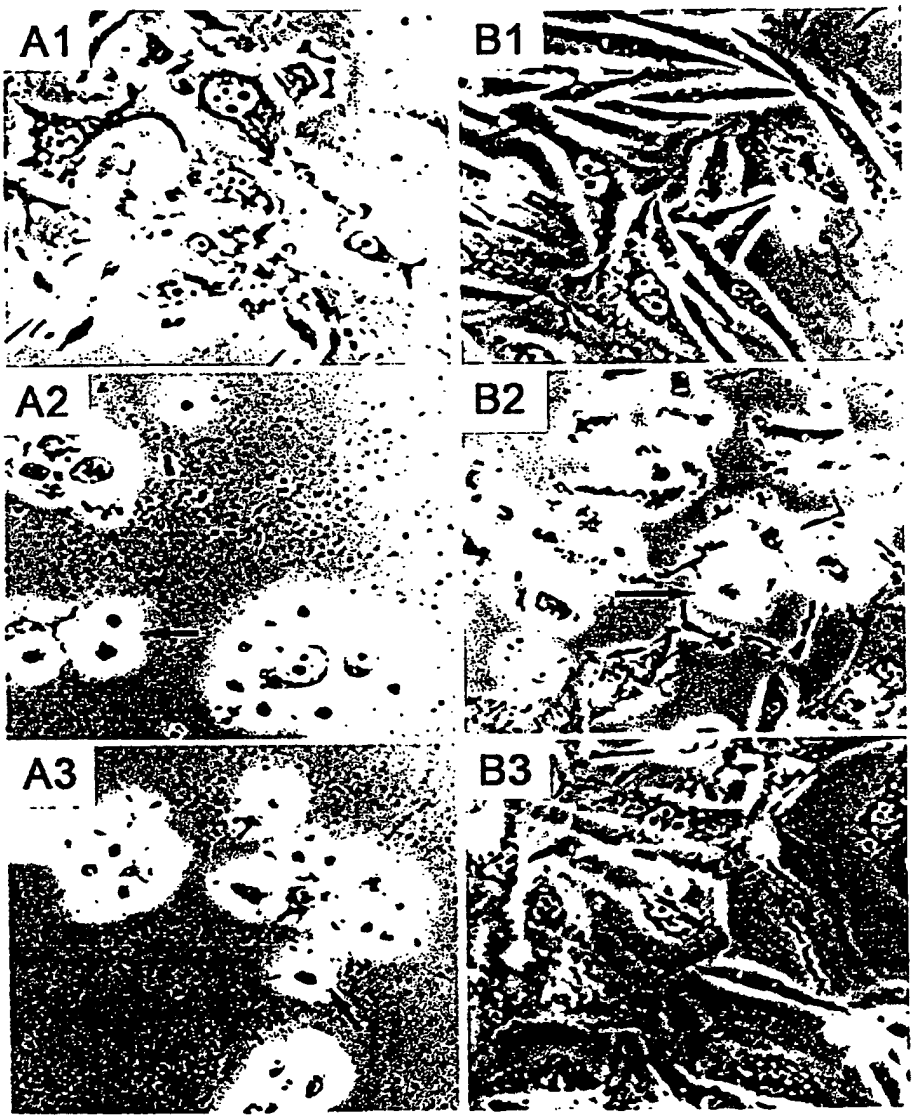


FIG.5

"Greg Morton"
<gmorton@agsales .com>
11/16/2005 04:48 PM

To "PAM LINCOLN" <pamela.lincoln@ipsen.com>
cc
bcc
Subject 940590-511

OOPS!

Pam,

When I loaded your shopping lists into the online catalog, I entered copy paper by the ream, instead of the Case. I just fixed it. However, on the above PO# 940590-511, it was entered as "6 reams @ $2.80 per ream, instead of 6 cases @ $28.00.
I fixed it on my end, but you will have to adjust your PO. The subtotal for the paper should be $168.00, not $16.80.

Sorry,

Greg Morton
A&G Sales